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ω-3 fatty acids attenuate glomerular capillary hydraulic pressure in rats with renal ablation

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ω-3 fatty acids attenuate glomerular capillary hydraulic pressure in rats with renal ablation. To clarify the emerging role of ω-3 fatty acids (FAs) in the regulation of the renal microcirculation, we recently performed micropuncture studies in normal rats maintained on diets enriched with ω-3 FAs. Although those studies suggested that ω-3 FAs alter the renal microcirculation in normal rats, it was not apparent whether this dietary maneuver could modulate intrarenal hemodynamics in the setting of renal disease. Therefore, the present renal micropuncture studies were performed in nephrectomized rats maintained on control diets or diets enriched with ω-3 FAs. ω-3 FAs abrogated glomerular capillary (56.2 ± 0.8 vs. 63.9 ± 2.0 mm Hg) and transcapillary hydraulic pressure (40.9 ± 1.4 vs. 50.6 ± 1.3 mm Hg) compared to untreated rats. This effect was attributable to (1) a reduction in mean arterial pressure (138 ± 3 vs. 163 ± 2 mm Hg) and (2) a decrease in efferent arteriolar resistance (0.43 ± 0.06 vs. 0.98 ± 0.19 dyn × seconds × cm^{-5} × 10^{10}). Sclerosis index and albuminuria were also lessened by this dietary maneuver. To further characterize the mechanism of altered renal arteriolar resistance, we then explored the effects of ω-3 FAs on renal prostaglandin synthesis and angiotensin II-stimulated phospholipid turnover. A significant decrease in the urinary excretion of the renal vasoconstrictor, TXA_2 (12.8 ± 2.3 vs. 35.1 ± 14.0 ng/24 hr), was induced by treatment with ω-3 FAs. Moreover, angiotensin II-stimulated phospholipid turnover was attenuated in intact glomeruli pretreated with ω-3 FAs. We conclude that ω-3 FAs exert favorable effects on experimental renal injury by eliciting a salutary effect on the renal microcirculation of rats subjected to subtotal renal ablation. Moreover, the similarities between these findings and those obtained with sustained inhibition of angiotensin II converting-enzyme suggest that these compounds act through parallel pathways of inhibition.

Although ω-3 fatty acids are well established as essential nutrients in developing humans and adults [1], their status as therapeutic agents in the management of various clinical conditions has only recently received attention [2, 3]. In spite of early enthusiasm for utilizing these agents in the management of progressive renal disease [3], hypertension [4], and atherosclerosis [5] a consensus regarding the role of ω-3 fatty acid supplementation in these conditions has yet to be established. Essential to proposing a utility for these agents in the management of disorders of the microcirculation, such as progressive renal disease, is an appreciation of the physiological implications of such manipulation. Importantly, recent studies from our laboratory have demonstrated that dietary consumption of ω-3 fatty acids can modulate the renal microcirculation in a pattern which parallels that observed after sustained inhibition of angiotensin II converting-enzyme. Specifically, increases in single nephron glomerular filtration rate (SNGFR) and single nephron plasma flow (Q_A) were observed in normal rats fed diets enriched with menhaden oil [6]. A reduction in total renal arteriolar resistance and, in particular, efferent arteriolar resistance was responsible for increased Q_A and SNGFR in this model. While several studies have suggested that ω-3 fatty acids can modulate intrarenal hemodynamics in experimental renal injury, those studies have failed to specifically examine glomerular hemodynamics following dietary enrichment with ω-3 fatty acids. Therefore, the purpose of the present study was to test the hypothesis that ω-3 fatty acids abrogate abnormal glomerular hemodynamics in the setting of renal disease. Accordingly, we chose to study the effects of ω-3 fatty acids in rats subjected to subtotal renal ablation, since this model is characterized by sustained glomerular capillary hypertension which can be abrogated by selective inhibition of angiotensin II [7, 8]. The studies reported herein demonstrate that administration of ω-3 fatty acid enriched diets lessened glomerular capillary hydraulic pressure (P_{GC}) and reduced renal arteriolar resistance in remnant nephrons. We then sought to determine the potential mechanisms which may contribute to these hemodynamic adjustments. Decreased intrarenal liberation of the potent renal vasoconstrictor TXA_2 and/or attenuation of angiotensin II-stimulated phospholipid turnover may each contribute to the hemodynamic effects of ω-3 fatty acids. An additional objective of these investigations was to assess the effects of ω-3 fatty acids on progressive renal injury, since previous investigations have suggested that diets enriched with ω-3 fatty acids may either retard or accelerate the rate of progressive glomerular injury following renal ablation [9–12]. Thus, long-term investigations of glomerular morphology and albuminuria were also carried out.

Methods

Experimental design

Study 1. An initial investigation was performed to characterize the effects of diets enriched with ω-3 fatty acids on glomerular hemodynamics in rats with renal ablation. In these studies 20 male Sprague-Dawley rats were subjected to subtotal renal ablation and randomly assigned to standard laboratory chow ($N = 10$) or chow enriched with 17% menhaden oil ($N = 10$) (ICN Biochemicals, Costa Mesa, CA, USA). Rats ($N = 10$) subjected to sham surgery served as controls. The diets were virtually isocaloric (4.45 vs. 4.63 Kcal/g diet in the control and menhaden oil enriched diet,

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respectively) and provided equivalent protein concentrations (20.8%). Linoleic acid (4.5% corn oil) was provided in sufficient quantities in each dietary group to prevent essential fatty acid deficiency. In addition, Tenox 20 (butylated hydroxytoluene; Eastman Kodak, Rochester, NY, USA), and α-tocopherol (0.33%) was present in each diet to inhibit autooxidation. Rats were pair-fed for a period of four to six weeks and then prepared for glomerular micropuncture studies as described below. Moreover, systemic tail-cuff blood pressure measurements were obtained at this time interval.

Study 2. Since the effects of ω-3 fatty acids on the renal microcirculation paralleled those observed following the administration of angiotensin II converting-enzyme inhibition we then wished to explore the effects of ω-3 fatty acids on angiotensin II-stimulated phosphatidylinositol hydrolysis, since hydrolysis of phosphatidylinositol with subsequent liberation of lipid second messengers is thought to modulate cell contraction and hence vascular resistance in the glomerulus [13, 14]. Six-week-old male Sprague-Dawley rats were fed standard laboratory chow or chow supplemented with 17% menhaden oil. Intact glomeruli were isolated after four to six weeks of dietary supplementation. Following a 45 minute stabilization period, glomerular suspensions were stimulated with 1×10^{-8} M angiotensin II. The incubations were terminated with iced methanol at early time intervals (< 2 min) and mass quantities of sn-1,2-diacylglycerol (sn-1,2-DAG) determined. Early intervals were chosen to reflect principally phosphatidylinositol hydrolysis rather than phosphatidylcholine degradation [15, 16]. In a separate group of rats lipid was extracted from glomeruli and analyzed for fatty acid composition.

Study 3. Since glomerular capillary hypertension is thought to evoke adverse structural changes in remnant glomeruli, we also wanted to examine the effects of ω-3 fatty acids on progressive glomerular destruction. Therefore, a long term investigation (10 weeks) to characterize the extent of glomerular injury in nephrectomized rats maintained on ω-3 fatty acids was carried out. The experimental groups and diets were identical to those described in study 1. Twenty-four hour urine collections for albumin excretion were obtained at three, six and nine weeks following subtotal renal ablation. In addition, glomerular morphometry was performed at the termination of the study. In order to confirm the authenticity of the diet administered and the potential interrelationship of lipid composition, renal injury, and prostaglandin turnover we also performed fatty acid analysis of the renal cortex at the termination of these studies. Blood was obtained at the termination of the experiment for serum albumin, total protein, cholesterol, triglycerides, urea nitrogen, and creatinine determinations. In addition, since ω-3 fatty acids have been shown to modulate dienoic prostaglandin metabolism we obtained 24 urine collections for prostaglandin metabolites at three, six, and nine weeks post-renal ablation.

Preparation of nephrectomized rats

Surgical reduction of renal mass was carried out as previously described [17]. Briefly, rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (Nembutal®). Under anesthesia, the left kidney was exposed with blunt dissection, and 2/3 infarcted by ligating branches of the renal artery. The right kidney was tied off and removed. After a thorough check for bleeding, the subcutaneous tissue was closed followed by a blind-stitch to the inner layer of the skin.

Micropuncture techniques

Rats were anesthetized with sodium pentobarbital (50 mg/kg body wt), placed on a temperature controlled operating table, and prepared for micropuncture as previously described [18]. Briefly, a tracheotomy was performed, the left femoral vein was cannulated (PE-50) and a bolus of Ringer's solution (0.5% body wt) was slowly administered over 15 minutes. Ringer's solution containing 25 μCi ^{3}H-inulin/ml was then infused at a rate of 0.5 ml/hr/100 g body wt for the remainder of the experiment. The femoral artery was cannulated (PE-50) and mean arterial pressure was monitored with a digital display pressure transducer. A bladder catheter (PE-50) was placed suprapubically. The left kidney was exposed by a subcostal incision, dissected free of perinephric tissue, immobilized in a plastic holder, and continuously bathed in mineral oil at 37.5°C. After a 45-minute stabilization period, urine was collected in preweighed tubes for 30 minutes. During this interval, three to four timed (3 to 4 min) proximal tubular fluid collections were obtained from randomly selected superficial nephrons to determine SNGFR. Samples of urine, plasma, and tubular fluid were added to scintillation cocktail, and radioactivity of the samples measured in a liquid scintillation spectrometer (Model LS 230; Beckman Instruments, Inc., Fullerton, CA, USA).

Intratubular hydraulic pressures were measured under free-flow conditions in one group of tubules. Proximal tubular stop-flow pressures were obtained in the first surface convolutions distal to Bowman's space, after blockage of the tubular lumens with Sudan Black mineral oil. Pressures were also determined in randomly selected efferent vascular welling points. All pressure measurements were performed with a servo-null micropressure system (W-P Instruments, Inc., New Haven, CT, USA). P_{GC} was calculated as the sum of the proximal tubular stop-flow pressure and arterial colloid osmotic pressure (COP). Plasma COP was determined directly utilizing a colloid osmometer (Model 4401, Wescor, Inc., Logan, UT, USA). Blood samples were taken from efferent vascular welling points and analyzed, together with an arterial sample, for afferent (C_A) and efferent (C_E) arteriolar protein concentration using the Micro-Lowry technique. Single nephron filtration fraction (SNFF) and Q_A were calculated as follows:

$$SNFF = 1 - \frac{C_A}{C_E}, Q_A = \frac{SNGFR}{SNFF}$$

The glomerular ultrafiltration coefficient (K_f) was calculated using an iterative method as previously described [16]. Renal afferent (R_A) and efferent (R_E) arteriolar resistance were calculated using the following relationships:

$$R_A = 7.692 \times 10^{10} \times (MAP - P_{GC}) \times (1 - Hct) \div Q_A$$

$$R_E = 7.692 \times 10^{10} \times (P_{GC} - P_E)$$

$$\div \left(\left(\frac{Q_A}{1 - Hct} \right) - SNGFR \right), P_E \text{ is efferent arteriolar pressure.}$$

Tissue fatty acid determinations

The fatty acid composition of renal cortical tissue was determined as previously described [19]. Briefly, one-half gram of fresh

or frozen tissue was placed in 5 ml of preheated (90 to 95°C) dilute (0.02 N) acetic acid and heated for 30 minutes, homogenized, and then centrifuged at 12,000 rpm for 15 minutes at 0°C. The pellet was washed with 5 ml of dilute acetic acid. The lipid was extracted from the pellet by rehomogenizing in chloroform/methanol (1:1, vol/vol) and centrifuging at 3,000 rpm for five minutes. The pellet was re-extracted with chloroform/methanol (1:1, vol/vol) and centrifuged. The pellet was then re-extracted a final time with chloroform/methanol (1:2) and the combined chloroform extracts were evaporated to dryness and the lipid dissolved in a known volume of chloroform. The methyl esters of the phospholipid and triacylglycerol fractions were transesterified using hexane.

The fatty acid methyl esters were analyzed on a Varian Model 3500 capillary gas chromatograph equipped with a flame ionization detector and a 30 meter capillary column (Supelcowax 10, Supelco, Inc., Bellefonte, PA, USA). The column temperature was programmed from 200°C to 225°C at 1.5°C/min increments with a final hold of 17 minutes. Fatty acid standards were obtained from Supelcowax (PUFA 1, PUFA 2, Supelco Inc., Bellefonte, PA, USA).

Glomerular morphometry

Renal tissue from rats was examined in a blinded manner using light microscopy (400 ×). Coronal sections were fixed in Zenker's solution, embedded in paraffin, and stained with periodic acid-Schiff (PAS) and Masson's trichrome stain.

Sclerosis index was determined based upon the method of Raij [20]. One hundred glomerular profiles were examined and assigned a semiquantitative score as follows: 0 is no sclerosis, 1 is ≤ 25% of the profile involved, 2 is > 25% but ≤ 50% of the profile involved, 3 is > 50% but ≤ 75% of the profile involved, and 4 is > 75% of the profile involved.

Glomerular areas were measured using a point counting method [21]. For each tissue specimen, 25 glomeruli encountered on a serpentine course between cortex and medulla were evaluated with a 100 point, calibrated, grid. The area of each glomerular cross-section was determined by multiplying the fraction of the points from the 100 point grid falling on the glomerular tuft by the area associated with each point. The glomerular area for each specimen was calculated as the mean of 25 individual measurements.

Mean glomerular volumes were determined by measuring the volume of at least 15 glomeruli using the Cavalieri principle [22]. The area was defined as the minimal convex polygon surrounding the capillary tuft estimated by superimposing a calibrated grid and counting the points falling on each profile. Only completely sectioned glomeruli were included in the analysis.

Prostaglandin determinations

Specific antibody to PGE_2 and TXB_2 (stable metabolite of TXA_2) was utilized to determine prostaglandin concentrations in urine. The antisera for these studies (Cayman Biologicals, Ann Arbor, MI, USA) has < 0.02% cross reactivity with other dienoic prostanoid metabolites. The enzyme immunoassay (EIA) system employed for these studies has excellent correlation ($r^2 = 0.99$) with gas chromatography/mass-spectrometry as previously described [23].

Urine aliquots (3 ml) were acidified to pH 3 to 3.5 with 0.1 N HCl acid and then passed through an octadecylsilica C_{18} column which had been activated with 10 ml methanol and washed with 10 ml water. The sample was then eluted with 10 ml of water, followed by 10 ml n-hexane and 10 ml ethylacetate. The ethylacetate fraction was taken to dryness under nitrogen, reconstituted with 0.5 ml EIA buffer and then subjected to EIA. Individual measurements of PGE_2 and TXB_2 were corrected for percent recoverability by adding a known quantity of ^{3}H-PGE_2 or ^{3}H-TXB_2 to the urine samples. Recovery ranged from 50 to 70%.

Urinary albumin excretion and serum chemistries

Twenty-four-hour urine collections were made with rats individually housed in metabolic cages. The urinary albumin concentration was determined utilizing an enzyme-linked immunoasssay system. (Exocell, Inc., Philadelphia, PA, USA).

Serum chemistries were performed colorimetrically using an autoanalyzer (Multistat III +, Instrumentation Laboratories, Lexington, MA, USA) as previously described [17].

Preparation of intact glomeruli

Following pentobarbital anesthesia the kidneys were perfused with 200 to 300 cc phosphate buffered saline (PBS) and then immediately removed and placed on ice. The renal cortex was trimmed, minced, and then passed through a series of steel mesh sieves of progressively smaller diameter (75, 150, 200 mesh). Glomeruli were then collected in Krebbs Henselheit (KH) and centrifuged at 3000 rpm × 30 minutes followed by resuspension in KH. The washing procedure was performed twice. Glomeruli were then treated with collagenase (type II, Sigma Chemical Co., St. Louis, MO, USA) and 0.03% DNase with gentle agitation (140 cycles/min) for 30 minutes to disrupt Bowman's capsule. After treatment the glomeruli were washed twice in KH and then resuspended in warm KH. Glomeruli were examined by light microscopy and deemed suitable for subsequent studies if > 90% purity was validated. After basal measurements of sn-1,2-DAG were obtained, angiotensin II (1×10^{-8} M) was administered and the reaction terminated at timed intervals (15 seconds, 30 seconds, 1 min, and 2 min) with iced methanol.

sn-1,2-DAG quantitation

Mass amounts of sn-1,2-DAG were quantitated using a modification of the assay developed by Priess et al [24]. Briefly, the sn-1,2-DAG's were extracted into chloroform and then converted to ^{32}P-phosphatidic acid utilizing *E. coli* sn-1,2-DAG kinase and ^{32}P-ATP of known specific activity. The amount of sn-1,2-DAG present in the sample was calculated from the amount of ^{32}P-phosphatidic acid generated.

Since *E. coli* sn-1,2-DAG kinase is stereospecific for sn-1,2-DAG, sn-1,2-DAG was quantitated as soon as possible after extraction to minimize acyl migration. The presence of sn-1,3-DAG and other nonreactive isomers was determined by thin layer chromatography. sn-1,2-DAG for use in generating a standard curve was prepared by digestion of 1,2-dioleoyl-sn-glycero-3PtdCh with phospholipase C from Bacillus cereus. The dioleoylglycerol generated from this hydrolysis was isolated by preparative silica gel G thin layer chromatography. The amount of standard sn-1,2-DAG was assayed by ester hydrolysis according to Stern and Shapiro [25].

Statistics

Data are expressed as the mean ± the SEM. All variables were screened using indices of kurtosis and skewness to determine whether the distribution was approximately Gaussian. When the

Table 1. Summary of renal microcirculation studies

Groups	Body wt *g*	Left Kidney wt *g*	Hct	MAP *mm Hg*	GFR[a] *cc/min*	P_{GC}	P_T	ΔP
						mm Hg		
ω-3 FAs (*N* = 8)	358 ± 13	1.42 ± 0.1[bc]	49 ± 1	138 ± 3[bc]	0.59 ± 0.07[b]	56.2 ± 0.8[c]	15.2 ± 0.6	40.9 ± 1.4[c]
SRA (*N* = 8)	341 ± 16	1.61 ± 0.1[b]	47 ± 1	163 ± 2[b]	0.54 ± 0.05[b]	63.9 ± 2.0[b]	13.4 ± 0.7	50.6 ± 1.3[b]
Control (*N* = 6)	347 ± 10	1.11 ± 0.1	48 ± 1	108 ± 4	2.16 ± 0.14	52.6 ± 1.6	13.7 ± 1.1	38.9 ± 1.5

Abbreviations are: FAs, fatty acids; SRA, subtotal renal ablation; Body wt, body weight; Left kidney wt, left kidney weight; Hct, hematocrit; MAP, mean arterial pressure; GFR, glomerular filtration rate; P_{GC}, glomerular capillary pressure; ΔP, transcapillary pressure; Q_A, single nephron plasma flow; SNGFR, single nephron glomerular filtration rate; R_A, afferent arteriolar resistance; R_E, efferent arteriolar resistance; K_f, ultrafiltration coefficient.

[a] Left kidney GFR for SRA rats versus 2-kidney GFR for controls

[b] $P < 0.05$ vs. control

[c] $P < 0.05$ vs. SRA

Table 1. *Continued*

Groups	Q_A	SNGFR	$R_A \times 10^{10}$	$R_E \times 10^{10}$	K_f
	nl/min		$d \cdot s \cdot cm^{-5}$		$nl \cdot s^{-1} \cdot mm\ Hg$
ω-3 FAs (*N* = 8)	414 ± 51[bc]	113 ± 14[b]	0.85 ± 0.08[b]	0.43 ± 0.06[bc]	0.085 ± 0.019[b]
SRA (*N* = 8)	294 ± 45[b]	96 ± 14[b]	1.65 ± 0.32	0.98 ± 0.19[b]	0.045 ± 0.006
Control (*N* = 6)	110 ± 7.5	32 ± 2	2.20 ± 0.37	1.54 ± 0.15	0.030 ± 0.000

mean of two groups was compared the Student's *t*-test was employed. When the mean of three groups was compared ANOVA was employed, followed by the Bonferroni-Dunn multiple comparison procedure. All statistical analyses were performed on a Macintosh computer using the SYSTAT statistical software package (SYSTAT, Evanston, IL, USA).

Results

Study 1

The administration of ω-3 fatty acid enriched diets to rats with renal ablation had several salutary effects on the renal microcirculation, as summarized in Table 1. Most striking was the attenuation of glomerular and transcapillary hydraulic pressure. Although, subtotal renal ablation evoked the expected adaptive increase in P_{GC}, ω-3 fatty acid supplemented diets restored P_{GC} to control values. ω-3 fatty acid enriched diets also restored transcapillary hydraulic pressure in rats with subtotal renal ablation. The mechanism responsible for ameliorating the adaptive increase in glomerular capillary pressure in this model was attributable to two factors. First, rats receiving diets enriched with ω-3 fatty acids demonstrated a 15% reduction in mean arterial pressure as compared to untreated nephrectomized rats. These effects were evident at the time of micropuncture and also in awake rats using the tail-cuff method (178 ± 11.4 vs. 148 ± 12.6 mm Hg). Second, ω-3 fatty acid administration significantly diminished efferent arteriolar resistance in nephrectomized rats. Although afferent arteriolar resistance was correspondingly reduced by ω-3 fatty acid enriched diets these trends did not achieve statistical significance ($P < 0.07$). The former effect is particularly important since a reduction in mean arterial pressure alone may not evoke a corresponding reduction in glomerular pressure in the absence of reduced efferent arteriolar tone [26]. SNGFR increased slightly ($P = 0.1$) by ω-3 fatty acid administration, largely as a consequence of an increase in Q_A. The latter was incident to a net decrease in total renal vascular resistance (2.63 ± 0.48 vs. 1.27 ± 0.08 days × second × cm^{-5} × 10^{10}). Although, the ultrafiltration coefficient appeared to increase after ω-3 fatty acid supplementation this trend did not achieve statistical significance ($P = 0.08$). Collectively, the hemodynamic effects observed after ω-3 fatty acid administration are reminiscent of those observed after angiotensin II blockade, suggesting a parallel mechanism of action.

Study 2

ω-3 fatty acid supplementation had a marked effect on angiotensin II-stimulated phospholipid turnover in freshly isolated glomeruli. Whereas sn-1,2-DAG peaked between 60 and 120 seconds in control glomeruli, no such increment was observed in glomeruli obtained from rats fed fatty acid enriched diets (Fig. 1A). Accordingly, sn-1,2-DAG mass approximately doubled in control glomeruli stimulated with angiotensin II, while little increase was observed in glomeruli obtained from rats previously maintained on diets enriched with ω-3 fatty acids (Fig. 1B). The fatty acid content of intact glomeruli (Fig. 2) was analogous to the profile obtained in whole kidney cortex (Fig. 4). Thus, menhaden oil increased the relative concentration of glomerular ω-3 fatty acids while diminishing the content of the ω-6 class of fatty acids.

Study 3

Glomerular injury was significantly lessened in nephrectomized rats maintained on diets enriched with ω-3 fatty acids. Specifically, albuminuria was reduced by 50 to 75% at each study interval (Fig. 3A). Urinary albumin excretion averaged 45.5 ± 9.7 mg/24 hr three weeks after subtotal renal ablation as compared to 16.5 ±

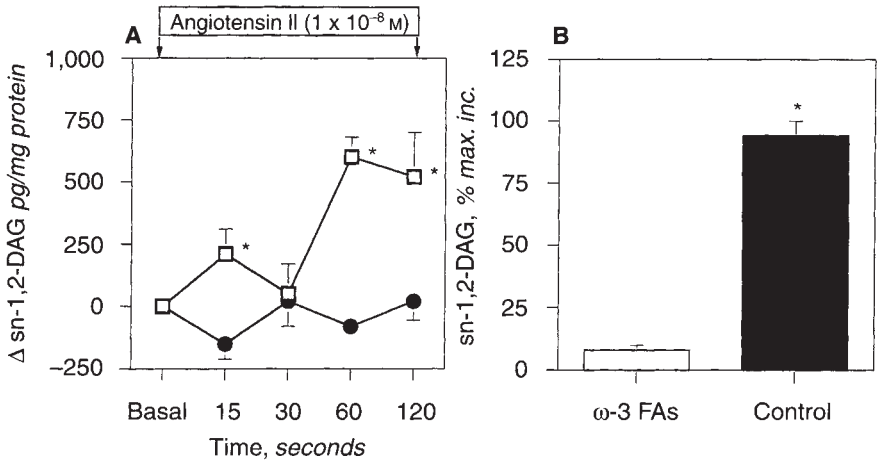

Fig. 1. *Angiotensin II-stimulated phospholipid turnover in suspensions of intact glomeruli.* **A.** Δ in sn-1,2-diacylglycerol (DAG) mass in control glomeruli (□) and glomeruli obtained from rats maintained on diets enriched with ω-3 fatty acids (●). $^*P < 0.05$. **B.** Percent maximal increase as a fraction of basal sn-1,2-DAG determinations obtained from control glomeruli or glomeruli isolated from rats fed diets enriched with ω-3 fatty acids. $^*P < 0.05$.

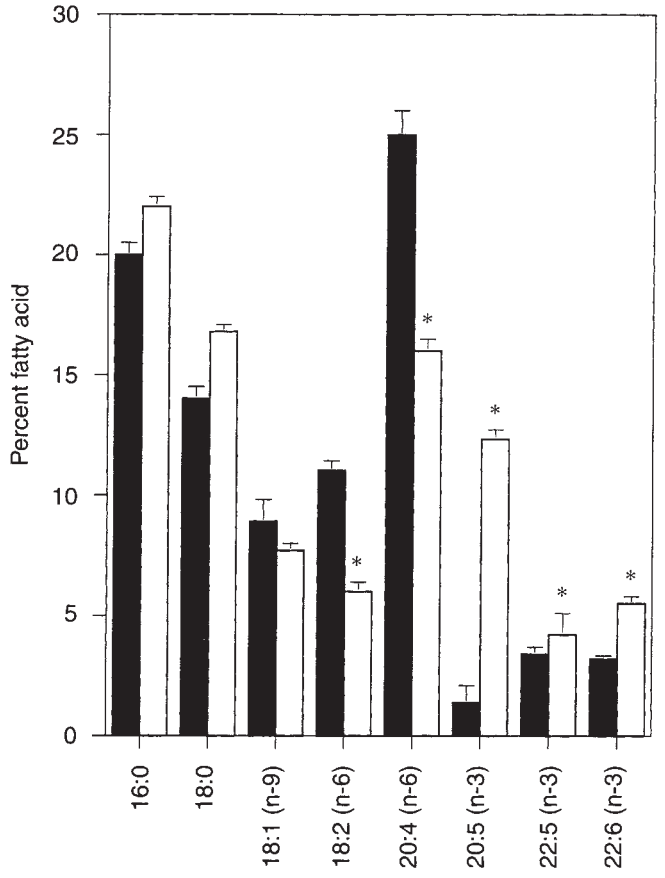

Fig. 2. *Percent fatty acid content of intact glomeruli.* Symbols are: (■) control glomeruli; (□) isolated glomeruli from rats maintained on diets enriched with ω-3 fatty acids. Abbreviations are: 16:0, palmitic acid; 18:0, stearic acid; 18:0 (ω-9), oleic acid; 18:2 (ω-6), linoleic acid; 20:4 (ω-6), arachidonic acid; 20:5 (ω-3), eicosapentaenoic acid; 22:5 (ω-3), docosapentaenoic acid; 22:6 (ω-3), docosahexaenoic acid. $^*P < 0.05$ vs. controls.

5.2 mg/24 hr in nephrectomized rats receiving diets enriched with ω-3 fatty acids. Albuminuria increased to 113.7 ± 8.6 mg/24 hr nine weeks after subtotal nephrectomy as compared to 60.7 ± 3.2 mg/24 hr in nephrectomized rats fed fatty acid enriched diets. As a corollary glomerular morphometry revealed a marked attenuation of sclerosis index in rats receiving ω-3 fatty acid supplementation (0.83 ± 0.2 vs. 0.20 ± 0.1; Fig. 3B). Indeed, the sclerosis index of treated rats was similar to two-kidney control rats. Glomerular growth was also reduced in treated rats as manifested by a reduction in mean glomerular area (7178 ± 462 vs. 6223 ± 499 m^2) and volume (0.77 ± 0.07 vs. 0.62 ± 0.08 m^3). Serum cholesterol and creatinine were increased in rats with renal ablation as compared to control rats or nephrectomized rats maintained on ω-3 fatty acids (Table 2). A significant decrease in total serum protein and albumin was also evident in untreated rats with renal ablation. Although ω-3 fatty acids may directly modulate lipid metabolism, the metabolic changes detailed above may also be consistent with attenuated renal injury and the attendant decrease in glomerular proteinuria.

Several changes in fatty acid content of the remnant kidney cortex were identified (Fig. 4). These included a 48% increase in oleic acid (18:1, ω-9) and a 15% decrease in the oleate progenitor, stearic acid (18:0). Depletion of the ω-6 fatty acids, arichidonate (20:4, ω-6) and linoleate (18:2, ω-6) by 10% and 25%, respectively, was also observed. These changes are consistent with previous studies from our laboratory in this model [17].

The results of renal cortical fatty acid analysis in rats maintained on ω-3 fatty acids were reflective of the diet administered to the animals. The most striking changes occurred in the polyunsaturated fatty acid fraction. In particular, eicosapentaenoic acid (20:5,ω-3) concentration increased from 1.66 ± 0.7% to 12.3 ± 0.4% in rats fed diets enriched with ω-3 fatty acids. Docosahexaenoic acid (22:6, ω-3) also increased significantly in rats maintained on diets enriched with ω-3 fatty acids (5.50 ± 0.31% vs. 4.05 ± 0.14% in treated and untreated nephrectomized rats, respectively). A similar albeit statistically insignificant increase in docosapentaenoic acid (22:5, ω-3) was also observed in rats fed ω-3 fatty acids. Chronic administration of ω-3 fatty acid enriched diets restored the oleate and stearate concentrations to normal in nephrectomized rats. However, ω-3 fatty acid administration did not restore the ω-6 fatty acid content to control values. Indeed, ω-3 fatty acids resulted in a further decrease in ω-6 fatty acid content in the nephrectomized rat. The latter phenomenon likely reflects a homeostatic attempt to maintain the overall unsaturation index of the kidney constant following the incorporation of highly unsaturated fatty acids of the ω-3 class [27, 28].

Renal prostaglandin excretion was also affected by the administration of diets enriched with ω-3 fatty acids. Importantly,

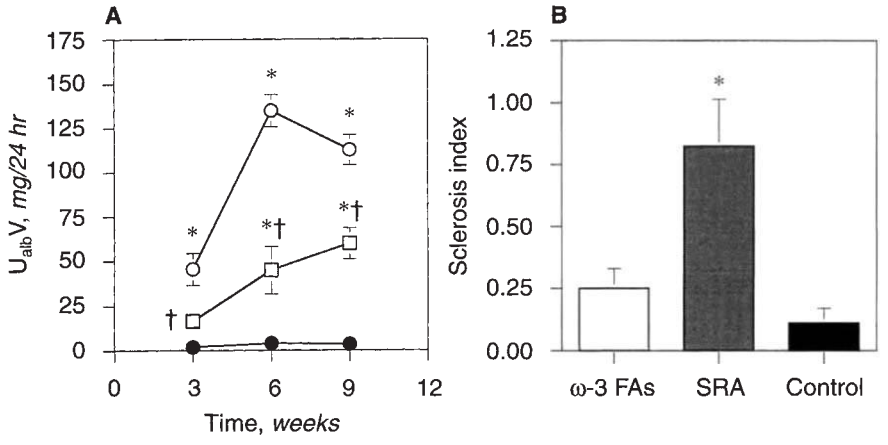

Fig. 3. *Indices of renal injury.* **A.** Twenty-four-hour urinary albumin excretion ($U_{Alb}V$) in control rats (●), or rats with subtotal renal ablation (SRA) maintained on standard chow (○) or chow enriched with ω-3 fatty acids (□). $^*P < 0.05$ vs. control; $\dagger P < 0.05$ vs. SRA. **B.** Sclerosis index of rats with subtotal renal ablation (SRA) versus control rats or SRA rats maintained on chow enriched with ω-3 fatty acids. $^*P < 0.05$ vs. other groups.

Table 2. Laboratory data of control rats, rats subjected to subtotal renal ablation (SRA) and SRA rats maintained on diets enriched with ω-3 fatty acids

Serum chemistries	Control	SRA	SRA + ω-3 FAs
Chol *mg/dl*	80.3 ± 5.4	112.9 ± 22.9[a]	98.2 ± 8.7[ab]
TG *mg/dl*	78.7 ± 4.4	61.2 ± 20.0	50.5 ± 5.3
BUN *mg/dl*	18.9 ± 1.0	33.5 ± 5.6[a]	34.1 ± 4.1[a]
Cr *mg/dl*	0.39 ± 0.0	0.92 ± 0.7[a]	0.75 ± 0.3[ab]
Alb *g/liter*	3.19 ± 0.5	1.95 ± 0.2[a]	2.35 ± 0.0[ab]
TSP *g/liter*	6.41 ± 0.2	4.45 ± 0.3[a]	5.08 ± 0.1[ab]

Abbreviations are: Chol, cholesterol; TG, triglycerides; BUN, blood urea nitrogen; Cr, creatinine; Alb, albumin; TSP, total serum protein.
[a] $P < 0.05$ vs. controls
[b] $P < 0.05$ vs. SRA

urinary excretion of the potent renal vasoconstrictor TXA_2 was reduced at each study period in rats maintained on fatty acid enriched diets compared to untreated rats (Fig. 5, left). Indeed, ω-3 fatty acid supplementation completely abrogated the expected increase in renal TXB_2 excretion. While PGE_2 excretion increased slightly in rats subjected to subtotal renal ablation, this trend did not achieve statistical significance. A slight, albeit statistically insignificant, attenuation of PGE_2 excretion was also observed in rats maintained on diets enriched with ω-3 fatty acids compared to untreated rats (Fig. 5B). These findings suggests that administration of ω-3 fatty acid enriched diets favorably alters the vasoconstrictor to vasodilator prostaglandin ratio in remnant nephrons and provides a possible clue to the underlying mechanism of arteriolar dilation following this dietary maneuver.

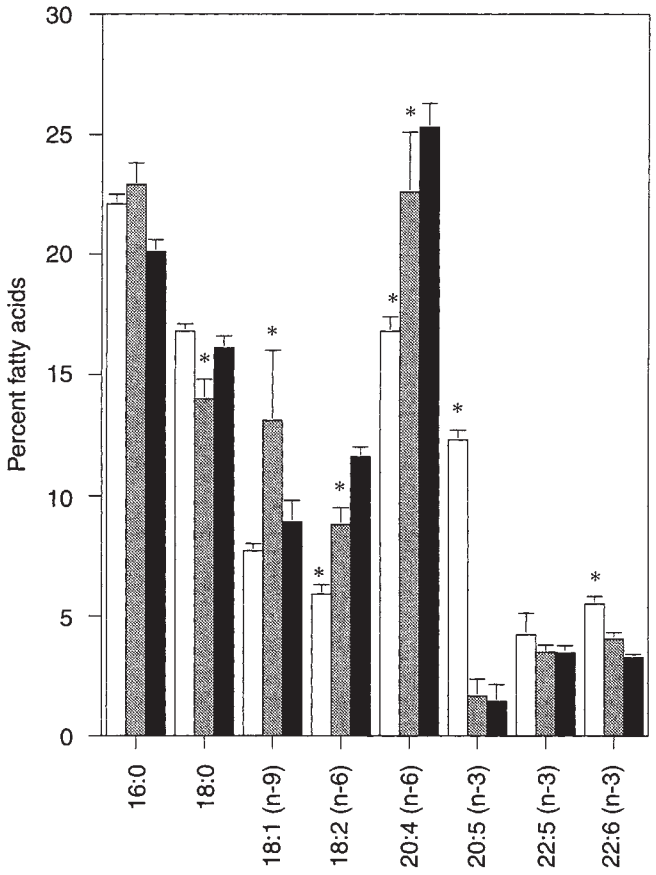

Fig. 4. *Percent fatty acid content of renal cortex.* Symbols are: (□) rats with subtotal renal ablation maintained on diets enriched with ω-3 fatty acid; (▨) rats with subtotal renal ablation on standard chow; (■) control rats. Abbreviations are: 16:0, palmitic acid; 18:0, stearic acid; 18:0 (ω-9), oleic acid; 18:2 (ω-6), linoleic acid; 20:4 (ω-6), arachidonic acid; 20:5 (ω-3), eicosapentaenoic acid; 22:5 (ω-3), docosapentaenoic acid; 22:6 (ω-3), docosahexaenoic acid. $^*P < 0.05$ vs. other groups.

Discussion

As anticipated, glomerular hyperfiltration, glomerular hyperperfusion, and glomerular capillary hypertension were obtained in rats subjected to subtotal renal ablation [8]. That such adaptive changes in nephron function serve to potentiate progressive renal injury has received extensive support and has advanced the concept that selective lowering of glomerular hydraulic pressure is an important therapeutic objective in the management of progressive renal disease. Thus, an important finding in the present investigation was the salutary effect of ω-3 fatty acid administration on P_{GC}. The beneficial effects of ω-3 fatty acids on intraglomerular pressure appeared to derive from at least two factors. First, dietary enrichment with ω-3 fatty acids reduced mean arterial pressure which, in turn, would effectively diminish pressure transmitted to the glomerular capillary network. And secondly, ω-3 fatty acids resulted in a significant reduction in efferent arteriolar resistance which would fundamentally dissipate glomerular pressure. The latter effect is particularly meaningful, since a

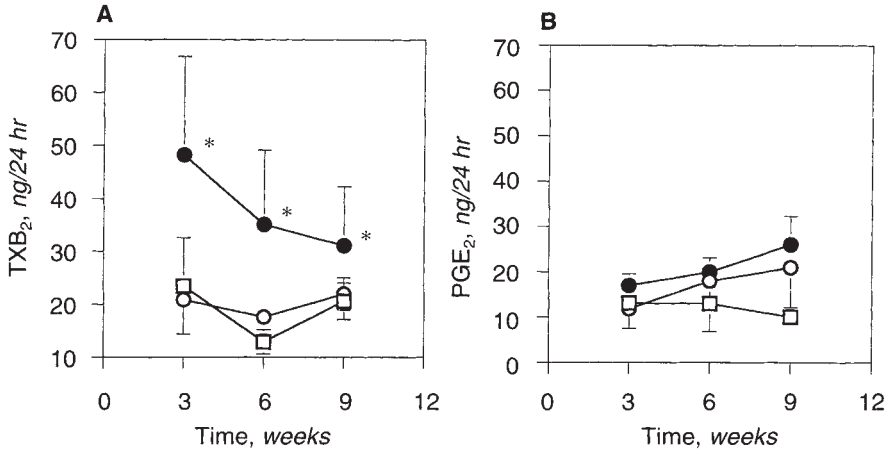

Fig. 5. *Twenty-four hour urinary excretion of dienoic prostaglandin metabolites.* **A.** TXB_2 excretion in control rats (□) versus nephrectomized rats maintained on standard chow (●) or chow enriched with ω-3 fatty acids (○). $^*P < 0.05$ versus other groups. **B.** PGE_2 excretion in control rats (□) versus nephrectomized rats maintained on standard chow (●) or chow enriched with ω-3 fatty acids (○).

reduction in mean arterial pressure *per se* is not necessarily accompanied by a fall in P_{GC} [26]. Indeed, the decrease in afferent arteriolar resistance observed in the present study would tend to facilitate the transmission of systemic pressure to the glomerular capillary bed and, hence, offset the expected decrease in glomerular pressure incident to diminished mean arterial pressure. Thus, antihypertensive agents which decrease efferent arteriolar resistance confer a selective advantage in the management of elevated glomerular pressure, particularly in the setting of subtotal renal ablation [29]. An increase in Q_A was also observed after dietary enrichment with ω-3 fatty acids, most likely attributable to a reduction of total renal arteriolar resistance induced by this dietary maneuver. SNGFR was only marginally increased after dietary enrichment with ω-3 fatty acids, presumably because of the offsetting effects of increased Q_A and K_F coupled with decreased P_{GC}. Importantly, while other strategies have been noted to diminish glomerular capillary pressure in remnant glomeruli, such as protein restriction, administration of L-arginine; the collective hemodynamic effects of ω-3 fatty acids on the renal microcirculation paralleled those obtained after sustained inhibition of angiotensin II converting-enzyme [7], thus supporting the hypothesis that ω- 3 fatty acids act on the renal microcirculation via a mutual pathway of inhibition.

Interference with the intrarenal synthesis of TXA_2 or blockade with a selective TXA_2 receptor antagonist ameliorates angiotensin II stimulated increases in renal vascular resistance [30], suggesting that angiotensin II elicits renal arteriolar vasoconstriction via a TXA_2 dependent pathway. Importantly, in our investigation, ω-3 fatty acid administration resulted in a marked decrease in the renal excretion of TXB_2 at each study period. Indeed, the renal excretion of TXB_2 at the time of micropuncture was 180% less in treated versus untreated rats. This finding offers one plausible explanation for the marked diminution in renal vascular tone after ω-3 fatty acid ingestion. Administration of ω-3 fatty acids in various clinical and experimental settings has commonly been associated with reductions in the liberation of TXA_2 [31–33]. Indirect evidence suggests that ω-3 fatty acids modulate dienoic prostaglandin synthesis by serving as a competitive substrate for cyclooxygenase. The latter effect is believed to favor the synthesis of the less potent trienoic thromboxane analog, TXA_3. Moreover, such a phenomenon is postulated to contribute to the antiplatelet and vascular effects of ω-3 fatty acids [34]. Since the antibody used to characterize TXB_2 excretion in the present study does not differentiate between dienoic or trienoic thromboxane metabolites, the assay employed will generally overestimate thromboxane derived from TXA_2. However, this shortcoming serves to strengthen the conclusion that a reduction in TXA_2 synthesis is obtained in rats fed diets enriched with ω-3 fatty acids. Whether ω-3 fatty acids modulate TXA_2 synthesis through other pathways of inhibition remains to be characterized. However, a preliminary report from our laboratory demonstrated no change in the transcript levels for TXA_2 synthase following the administration of ω-3 fatty acids to nephrectomized rats [35]. This finding suggests that ω-3 fatty acids exert their actions on TXA_2 synthesis via post-transcriptional modification(s) of TXA_2.

Interestingly, we did not observe a significant change in the urinary excretion of PGE_2 in remnant rats treated with ω-3 fatty acid supplementation. Since the antisera utilized in this study exhibits 100% cross-reactivity with PGE_3, it is possible that the urinary excretion of PGE_2 is substantially less than that measured by our assay. In this regard, one report demonstrated a significant decrement in urinary PGE_2 in rats fed diets enriched with fish oil [12], while other laboratories have shown little change in urinary PGE_2 excretion after dietary administration of ω-3 fatty acids [11]. It is likely that utilization of PGE_2 antisera with variable cross-reactivity to PGE_3 contributes to disparate results among different laboratories. Indeed, the dissimilar results of previous studies coupled with variable antisera cross-reactivity precludes precise understanding of the biological significance of changes in measured PGE_2. However, it is worthwhile noting that the biological potency of PGE_3 is believed to be similar to that of PGE_2 [33]. This contrasts sharply with the arguments presented above for the trienoic metabolites of thromboxane and suggests that, overall, dietary administration of ω-3 fatty acids results in a relative increase in the vasodilator/vasoconstrictor prostaglandin ratio.

An equally plausible explanation for the actions of ω-3 fatty acids on the renal microcirculation of nephrectomized rats can be inferred from studies demonstrating diminished binding of angiotensin II to adrenal zona glomerulosa and smooth muscle cells following pretreatment with polyunsaturated fatty acids [36, 37]. Thus, ω-3 fatty acids could exert an inhibitory effect on angiotensin II receptor coupled intracellular signaling events [37, 38] including the production of lipid second messengers [39]. Lipid second messengers are believed to contribute to the constriction of rat renal microvessels [40, 41]. In a preliminary report, ω-3 fatty acid administration resulted in a blunted renal vasoconstrictor

response to angiotensin II in the renal vasculature of the rat [42]. Dietary fish oil supplementation in humans has also been associated with a reduced pressor response to exogenous angiotensin II [43]. Accordingly, we examined angiotensin II-stimulated phospholipid turnover in the intact glomerulus following chronic dietary administration of ω-3 fatty acids. Chronic pretreatment of normal rats with ω-3 fatty acids resulted in a marked diminution in the liberation of sn-1,2-DAG following administration of angiotensin II. This finding suggests that chronic dietary administration of ω-3 fatty acids inhibit angiotensin II-stimulated membrane lipid turnover in the intact glomerulus. Thus, it is possible that altered angiotensin II stimulated intracellular release of lipid second messengers contributes to arteriolar vasodilatation following dietary enrichment with ω-3 fatty acids. Whether similar findings occur in glomeruli obtained from rats subjected to subtotal renal ablation cannot answered by the present studies. However, glomeruli obtained from remnant rats contain varying degrees of mesangial expansion and glomerulosclerosis resulting in inconsistent phospholipid turnover and, therefore, disparate findings (unpublished observations).

An important additional finding in the present investigation was the attenuation of renal injury incurred by dietary enrichment with ω-3 fatty acids. Both proteinuria and glomerulosclerosis were lessened by dietary administration of ω-3 fatty acids. Glomerular growth, as manifested by glomerular area and volume were correspondingly reduced after dietary supplementation with ω-3 fatty acids. Importantly, a beneficial effect of ω-3 fatty acid enriched diets on glomerular structure and function has been reported in three previous studies in the remnant model of renal injury [9–11]. In contrast, one study in the remnant model of experimental renal injury [12] has demonstrated a deleterious effect of fish oil administration on renal structure and function. The latter study differed from previous studies, and our own, by the omission of essential fatty acid supplements and antioxidants from the fatty acid enriched diets [10]. Importantly, omission of antioxidants may allow highly unsaturated fatty acids to undergo oxidation while in storage. Subsequent ingestion of oxidized lipids may then result in direct tissue injury and contribute to early mortality and loss of renal function. Although the mechanism responsible for attenuating glomerular injury in remnant rats fed diets enriched with ω-3 fatty acids remains uncertain, we hypothesize that the reduction in glomerular hydraulic pressure obtained in the present study exerts a beneficial effect on progressive renal injury [29, 44].

An additional factor which may have contributed to progressive glomerular destruction in this study was the increase in circulating cholesterol observed in rats with advancing renal disease [45]. Since dietary enrichment with ω-3 fatty acids was associated with a reduction in circulating cholesterol, it is possible that these agents exerted a beneficial effect on progressive glomerular injury through modulation of lipid metabolism. Such a lipid lowering effect is well described following dietary enrichment with ω-3 fatty acids [2, 5]. However, ω-3 fatty acids typically result in striking decreases in circulating triglycerides, whereas the effects of these compounds on serum cholesterol are generally minor. Therefore, we suspect that the modest effects of ω-3 fatty acids on serum cholesterol in the present investigation were secondary to the beneficial effects of ω-3 fatty acids on glomerular proteinuria rather than a metabolic effect *per se* induced by this dietary maneuver. Regardless, the lipid lowering effect of ω-3 fatty acids in the setting of renal disease may subsequently elicit a favorable effect on the course of progressive glomerular destruction.

Several changes in cortical fatty acids were observed in nephrectomized rats with advancing renal disease. The most striking alterations included a fall in linoleic, arachidonic, and stearic acid concentration and a rise in oleic acid concentration. We have recently described these changes in detail and have coupled these events to increased endogenous turnover of arachidonic acid metabolites in the remnant kidney [17]. ω-3 fatty acid administration abrogated the changes in oleic and stearic acid content in nephrectomized rats, a finding consistent with previous studies from our laboratory demonstrating amelioration of these long chain fatty acid changes incident to diminished arachidonic acid turnover [17]. In contrast, the decrement in ω-6 fatty acid content was exacerbated by ω-3 fatty acid administration. Although, these data appear to conflict with our earlier report [17], the interaction of dietary ω-3/ω-6 intake reflect metabolic selectivities which maintain highly unsaturated fatty acid composition in tissue lipids constant [27]. Such a phenomenon is also in accord with studies examining the physical properties of cell membranes, such as membrane fluidity [46, 47]. Importantly, this phenomenon may contribute to the decline in TXB_2 excretion observed in rats fed ω-3 fatty acids since the availability of precursor (arachidonic acid) fatty acids would necessarily limit the synthesis of dienoic prostaglandins. Thus, the fatty acid changes induced by ω-3 fatty acids in this setting likely represent a series of biochemical adjustments to alterations in eicosanoid synthesis, membrane physical properties, and renal injury *per se*.

In summary, dietary enrichment with ω-3 fatty acids abrogated glomerular capillary hypertension and lessened renal injury in the remnant model of experimental renal disease. The mechanism(s) responsible for the attenuation of intraglomerular hypertension in rats treated with ω-3 fatty acids included a salutary effect on mean arterial pressure and a decrease in efferent arteriolar resistance. ω-3 fatty acids may exert these hemodynamic effects by reducing angiotensin II-dependent signaling events, such as phospholipid hydrolysis or by limiting the synthesis of the potent renal vasoconstrictor, TXA_2. The latter findings coupled with the effects of these agents on glomerular injury are reminiscent of those observed following the administration of agents which block angiotensin II converting-enzyme, and thus support the notion that these compounds act through a parallel pathway. Regardless, these studies furnish a physiological rationale which could potentially be applied to the treatment of progressive renal disease. Indeed, the favorable effects of ω-3 fatty acids on renal hemodynamics reported in this study may have surreptitiously contributed to the salutary outcome in recent clinical trials in which fish oil was administered to patients with IgA nephropathy and recipients of renal allografts [48, 49].

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